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Kateryna Toganov

*Philadelphia College of Osteopathic Medicine*

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# **Is Bovine Lactoferrin Effective in Preventing Diarrhea in Infants and Toddlers?**

Kateryna Toganov, PA-S

A SELECTIVE EVIDENCE BASED MEDICINE REVIEW

In Partial Fulfillment of the Requirements For

The Degree of Master of Science

In

Health Sciences – Physician Assistant

Department of Physician Assistant Studies  
Philadelphia College of Osteopathic Medicine  
Philadelphia, Pennsylvania

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## ABSTRACT

**Objective:** The objective of this selective evidence-based medicine (EBM) review is to determine whether bovine lactoferrin is effective in preventing diarrhea in infants and toddlers.

**Study design:** Review of three English-language primary randomized controlled trials published between 2007 and 2016.

**Data sources:** Three double-blind controlled trials comparing bovine lactoferrin to placebo were found using PubMed.

**Outcomes measured:** Incidence and incidence rate of diarrhea in episodes/child/year were measured using the information provided by the caregivers and the healthcare records obtained from a hospital or clinic.

**Results:** King et al. found that there was no statistically significant difference in incidence of diarrhea between the infants taking bovine lactoferrin (1.31 episodes/infant/year) versus placebo (1.35 episodes/infant/year) in a 0- to 12-months-old age group. Similarly, Ochoa et al. determined no statistically significant difference in diarrhea occurrence between children who received a bovine lactoferrin supplement (5.43 episodes/child/year) and the children who received placebo (5.15 episodes/child/year) in a 12- to 18-months-old age group ( $p = 0.375$ ). In contrast, Chen et al. found a significantly lower incidence rate of diarrhea in infants after exposure to bovine lactoferrin as compared to placebo (rate ratio of 0.32 with  $p < 0.05$ ) in a 4- to 6-months-old age group.

**Conclusion:** The evidence regarding whether bovine lactoferrin is effective in preventing diarrhea in infants and toddlers is conflicting. It appeared to be clinically effective in 4- to 6-months-old infants who were followed over a 3-months period, but had shown no effect in 0- to 12- and 12- to 18-months-old infants and toddlers who were followed for 12 and 6 months, respectively. Therefore, it is possible that the effectiveness of bovine lactoferrin demonstrated by Chen et al. could have been influenced in some way by the short duration of the study and by the especially narrow age group.

**Key words:** bovine lactoferrin, diarrhea

**Introduction:**

Diarrhea is the passage of liquid or loose stools 3 or more times per day.<sup>1</sup> Worldwide, it is a leading cause of death among children younger than 5 years old and it carries a significant morbidity with substantial negative effects on population health.<sup>2</sup> In the United States, infectious diarrhea is most commonly caused by viral pathogens, such as rotavirus or norovirus and accounts for greater than 1.5 million outpatient visits and approximately 200,000 hospitalizations each year.<sup>3,4</sup> In 2015 in the United States, 140,000 cases of diarrheal disease were reported in children younger than 5 years old, with 293 deaths.<sup>5</sup> The estimated direct medical cost of diarrheal-disease-related hospitalizations in the United States in 2013 was \$226 million with 70,553 hospitalizations in children younger than 5 years old.<sup>6</sup>

There is a number of etiologies that can cause diarrhea, including infectious ones, such as enteric adenovirus of serotypes 40 and 41, *Aeromonas* spp, *Entamoeba histolytica*, *Campylobacter* spp enteritis, cryptosporidiosis, typical enteropathogenic *Escherichia coli*, enterotoxigenic *E. coli*, norovirus, non-typhoidal *Salmonella* spp, rotaviral enteritis, shigellosis, cholera, and *Clostridium difficile*. Diarrhea can quickly lead to dehydration, and the following physical-exam findings may suggest its occurrence: tachycardia, delayed capillary refill, dry mucous membranes, decrease in urine output, reduced skin turgor, and decrease in body weight by 5% or more. The following methods of diarrheal prevention are currently recommended by the World Health Organization: having access to clean drinking water and using improved sanitation; promoting good hand, personal, and food hygiene; educating about the spread of infection; emphasizing the importance of exclusive breastfeeding for the first 6 months of an infant's life; and receiving rotavirus vaccine.<sup>1</sup>

Lactoferrin (LF) is a bilobed, iron-binding glycoprotein found in human breast milk, saliva, tears, intestinal and genital secretions, and secondary granules of neutrophils.<sup>7,8</sup> It has antimicrobial, anti-inflammatory, and immunomodulatory activities and has been shown to decrease virulence of enteropathogens in vitro.<sup>7</sup> The antimicrobial activity of LF includes sequestering iron that is necessary for bacterial growth and disrupting the integrity of the bacterial cell membrane.<sup>8</sup> Human LF and bovine lactoferrin (bLF) have similar structures, functions, and bioactivity, and bLF has been shown to be safe in infants.<sup>8</sup> Introducing bLF as a supplement for infants and toddlers may be an effective preventative measure for common diarrheal diseases.

**Objective:**

The objective of this selective evidence-based medicine (EBM) review is to determine whether bLF is effective in preventing diarrhea in infants and toddlers.

**Methods:**

The research was done by the author of this EBM review, and the databases used were PubMed and Cochrane Library. The key words used in the searches were “bovine lactoferrin” and “infant.” The English-language articles selected were published in peer-reviewed journals. They were selected based on their relevance to the question asked and on their assessment of patient-oriented outcomes (POEMs). Inclusion criteria were studies that were randomized controlled trials (RCTs) published from 2007 to 2017 and not involved in Cochrane database systematic reviews or meta-analyses. Exclusion criteria were studies that evaluated populations older than 3 years or premature infants. The statistics reported in the selected studies were *t* test, Poisson test, *p* value, and negative binomial linear regression models.

The population evaluated in these studies included infants and toddlers of ages ranging from 0 to 18 months. The three randomized, double-blind, placebo-controlled trials used in this review studied the effect of bLF supplementation on patients of this age group. Chen et al. administered either a 35.8 mg dose of bLF per day to an experimental group or a visually matched placebo to a control group, while using an exclusively breastfed group of infants as a reference. The trial lasted for 3 months. In a study by King et al. patients in a treatment group were given Similac formula supplemented with 850 mg/L of bLF, and patients in a comparison group received the same Similac formula, but with 102 mg/L of bLF in it. The intervention lasted for 12 months. Ochoa et al. administered either 1,000 mg/day of bLF or placebo over a period of 6 months. The outcome of interest included the effectiveness of bLF as a preventative measure in decreasing the incidence of diarrhea in infants and toddlers.

**Table 1:** Demographics and Characteristics of Included Studies

Study	Type	#Pts	Age (mths)	Inclusion Criteria	Exclusion Criteria	W/D	Interventions
Chen <sup>8</sup> (2016)	RCT	359	4-6	Apparent good health, Hb > 60 g/L, CRP < 10mg/L, parent/guardian approval for participating in all aspects of study and their agreement to avoid using other iron-fortified formulas or foods	History of severe, persistent, or chronic diarrhea; severe malnutrition; severe infections; serious chronic illness; personal/family history of allergy to cow's milk or infant formula; eczema; allergic rhinitis; or asthma	43	35.8 mg/day dose of bovine lactoferrin in infant formula
King <sup>7</sup> (2007)	RCT	79	0-12	Healthy infants 0-4 weeks old, born at >34 wk of gestation and weighed >2000g, strictly bottle fed, staying in area for	Intolerance to cow's milk formula, major congenital anomalies, immunodeficiency, HIV-infected	27	850 mg/L of bovine lactoferrin in infant formula

				at least 1 year, guardians had access to telephone	mother, parental inability to follow protocol		
Ochoa <sup>9</sup> (2013)	RCT	555	12-18	Previously weaned 12-18-mo children	History of severe, persistent, or chronic diarrhea; severe malnutrition; severe infections; serious chronic illness; personal/family history of allergy to cow's milk or infant formula; eczema; allergic rhinitis; or asthma	65	1,000 mg/day of bovine lactoferrin as a food supplement

### **Outcomes measured:**

Chen et al.<sup>8</sup> measured the effectiveness of bLF as compared to placebo in decreasing morbidity of diarrhea in 4- to 6-months-old infants. Caregivers were given a standardized checklist of symptoms to refer to when reporting an occurrence of a morbidity episode. They were requested to notify healthcare workers if their infants had exhibited any of the symptoms from the list, including diarrhea. Data were analyzed using negative binomial regression models that estimated the incidence rate of diarrhea, thereby evaluating the effectiveness of bLF in decreasing its morbidity. The models were adjusted for the covariables of age and sex.

Ochoa et al.<sup>9</sup> measured the effect of bLF on diarrheal prevention in 12- to 18-months-old toddlers. Previously trained community health workers performed daily home visits to administer coded bLF or placebo and to collect data on occurrence of diarrhea. Data were analyzed using Poisson test and Student *t* test to measure the incidence rate of diarrhea. King et al.<sup>7</sup> measured the effect of bLF on occurrence of diarrhea in infants 0 to 12 months old. The caregivers were contacted every 1 to 2 weeks via telephone or personally to inquire about any symptoms of

diarrhea in their infants. The diagnosis of diarrhea was made by a pediatric nurse practitioner based on parental recollection and medical records from an attended clinic or hospital. Student  $t$  test was used to analyze the data and compare the incidence rate of diarrhea between infants who received bLF and those who received placebo.

### **Results:**

Three studies compared the effect of bLF to placebo in infants and toddlers. Chen et al.<sup>8</sup> conducted their randomized controlled double-blind study in Chengdu City in western China from March 2012 to March 2013. They recruited 260 full-term infants 4 to 6 months of age who had been previously exclusively breastfed and were switching to formula feeding at that time. Additionally, 130 exclusively breastfed infants were recruited at the same time to be followed as a reference group. Inclusion and exclusion criteria are listed in Table 1. Three hundred and ninety infants met the inclusion criteria, and 31 were excluded because of their caregivers' refusal to enroll in the study. Formula that contained 38 mg/100 g of bLF was randomly assigned to eligible infants, and the average daily bLF intake was estimated to be 35.8 mg/day. The control group received bLF-free formula that was comparable in its nutrient content to the bLF-fortified formula. Both formulas were provided for free.

Compliance with the formula was evaluated using data from recording tables on which caregivers included the number of spoons of formula their infants consumed per day. Field workers distributed both formulas, supervised their consumption, and visited the families every weekend to evaluate the amount of formula intake. Over the course of the study, 5 of the infants were rejected because their caregivers used other formula. Caregivers of 22 infants lost the recorded data, and caregivers of 15 breastfed infants were supplementing with formula, thus leading to their subsequent rejection. One infant was lost to the study as a result of formula



allergy. Altogether, these losses represented 12% of the sample size and were reported as dropouts.

The incidence of diarrheal episodes per 100 child days in the bLF group was 0.57, whereas in the control group the incidence was 0.90; the number of diarrheal events was 59 and 79, respectively (Table 2). The reference group of exclusively breastfed infants had 55 diarrheal events, and the incidence of diarrheal episodes was 0.60. The rate ratio of diarrheal occurrence between infants who received bLF and infants who received placebo was 0.32, with a 95% CI (0.20 - 0.72) and  $p < 0.05$  (Table 3). The rate ratio between the breastfed group and the control group was 0.30, with a 95% CI (0.18 - 0.69) and  $p < 0.05$ . Finally, the rate ratio between the bLF group and breastfed group was 1.12, with a 95% CI (0.71 - 1.89) and  $p < 0.05$ . These values illustrate a significant difference between the bLF and control groups and no significant difference between the bLF and breastfed groups of infants.

**Table 2.** Incidence of Diarrheal Episodes from Chen et al.<sup>8</sup>

Groups	Diarrhea events	Incidence/100 child days
bLF	59	0.57
Control	79	0.90
Breastfed	55	0.60

**Table 3.** Rate Ratios of Diarrheal Occurrence from Chen et al.<sup>8</sup>

Groups	Rate ratio	95% CI	$p$ value
bLF/Control	0.32	(0.20 - 0.72)	$< 0.05$
Breastfed/Control	0.30	(0.18 - 0.69)	$< 0.05$
bLF/Breastfed	1.12	(0.71 - 1.89)	$> 0.05$

Ochoa et al.<sup>9</sup> conducted their community-based, randomized, double-blind, controlled trial in Lima, Peru, from January 2008 to May 2011. They included 555 and excluded 3,119 toddlers 12 to 18 months of age. The inclusion and exclusion criteria are included in Table 1.

Some toddlers were excluded because they were still being breastfed, caregivers of others refused to participate, and some fell under the exclusion criteria. Community health workers visited each family 6 days a week, twice a day to administer coded preparations of bLf or placebo and thereby to ensure compliance with the protocol. The participants consumed 0.5 g of bLF or placebo diluted in 25 mL of water 2 times a day.

Between both groups, 32 children were noncompliant with protocol and were excluded from the study. From the bLF and placebo groups respectively, 25 and 40 children dropped out for a number of reasons. The compliance of the study was estimated to be 92% for administered doses, 90% for monthly clinic visits, and 98% for home visits. The incidence rate of diarrhea was 5.4 and 5.2 episodes/child/year in the bLF and control groups, respectively (Table 4). The rate ratio for diarrhea occurrence between children who received bLF and children who received placebo was 1.05, with 95% CI (0.94 - 1.18) and  $p = 0.375$ . Therefore, no significant difference in incidence of diarrhea was noted between the bLF and control groups.

**Table 4.** Incidence Rates and Rate Ratio of Diarrheal Occurrence from Ochoa et al.<sup>9</sup>

Groups	Incidence rate (episodes/child/year)	95% CI	<i>p</i> value
bLF	5.43	(5.02 - 5.86)	N/A
Control	5.15	(4.74 - 5.59)	N/A
Rate ratio/comparison	1.05	(0.94 - 1.18)	0.375

King et al.<sup>7</sup> recruited their participants from newborn nursery and from outpatient clinic at the University of Maryland Medical System in Baltimore. They excluded infants with intolerance to cow's milk formula, with major congenital abnormalities, with immunodeficiency, with HIV-infected mothers, or with caregivers unable to follow the protocol (Table 1). Seventy-nine infants who were 0 to 4 weeks old, weighed >2,000 g, and were strictly bottle-fed enrolled in the study and were randomized to receive powdered Similac formula with or without added

bLF. The intervention and comparison preparations contained 850 mg/L and 102 mg/L of bLF, respectively. Both groups received formulas free of charge.

The caregivers received formula every 1 to 2 months, and its distribution was recorded to monitor compliance. Compliance with formula intake was found to be excellent. Approximately 33 oz of mixed formula per day were consumed by participants from both groups throughout the year. However, 27 of 79 infants dropped out from the study for a variety of reasons, and 13 of those who dropped out were from bLF group. No significant difference in incidence of diarrhea was noted between bLF and comparison groups. The episodes of diarrhea per infant-year were 1.31 and 1.35 in the bLF and comparison groups, respectively (Table 5). All three studies found bLF to be safe in infants and toddlers, and no adverse events or intolerance to its effects were observed in any of the participants.<sup>7-9</sup>

**Table 5.** Incidence of Diarrhea from King et al.<sup>7</sup>

Groups	Episodes/infant-year
bLF	1.31
Control	1.35

## **Discussion:**

The immunomodulatory, antibacterial, and antiviral effects of LF have been studied multiple times in the past with both bovine and recombinant human analogs. A systematic review from Cochrane Library concluded that bLF might be effective in preventing late-onset sepsis and necrotizing enterocolitis in neonates, although it considered the evidence to be of low quality.<sup>10</sup> The Food and Drug Administration (FDA) found both recombinant human LF and bovine LF to be safe for consumption.<sup>11,12</sup>

Two of the three studies determined that bLF had no significant effect on diarrheal prevention in the evaluated age groups.<sup>7,9</sup> King et al.<sup>7</sup> conducted their trial with a relatively small

number of participants and evaluated a large number of variables/outcomes, possibly leading to an occurrence of Type II error. Additionally, because the caregivers were contacted once every 1 to 2 weeks, they could have forgotten to report some episodes of diarrhea to the researchers. King et al.<sup>7</sup> did not provide *p* values or 95% CIs in their study, thus leading to questions regarding the quality of their evidence.

Although Ochoa et al.<sup>9</sup> conducted the study with a relatively large sample size, they also evaluated a significant number of variables/outcomes. Another limitation was the use of a single dose of bLF (1,000 mg/day), potentially too low for that age group.<sup>9</sup> Frequent home visits and health monitoring of participants might have improved their health risks and resulted in decreased incidence rates of diarrhea than it would have been in the absence of the study. The age range of the participants might have been too narrow, and different results might have been possible if the study included other age groups. Lastly, enteropathogens in Peru were different from those in some other parts of the world, and the frequency of children's exposure to them might have increased pathogen-specific immunity and led to lessening of effects from bLF.<sup>9</sup>

Chen et al.<sup>8</sup> found bLF to be effective in diarrheal prevention and demonstrated a statistically significant difference in incidence rate of diarrhea between bLF and placebo groups, as well as almost identical results between bLF and breastfed groups. Limitations of the study had some similarities to those in Ochoa et al., such as narrow age range of participants; only one dose of bLF tested (35.8 mg/day), which was potentially too low; and frequent home health educational interventions possibly modifying risks of diarrhea. Additionally, some bias might have been introduced in two of the studies, because formula was provided for free.<sup>7,8</sup> Lastly, the participants who did not follow the rules of the study were subsequently excluded from the analysis of the data and reported as dropouts, possibly causing the final results to appear better

than they would have been. Performing a worst-case analysis of the data would have been a more honest way to evaluate the outcomes.

As for the limitations of this EBM review, few randomized, double-blind, controlled trials that evaluate the effectiveness of bLF on diarrheal prevention are currently available. Also, age groups and doses of bLF vary significantly among the studies that are included in this review. Additionally, the differences in metabolism and in physiological development of newborns and 18-month-old infants are substantial and are important to take note of when answering the proposed question.

### **Conclusion:**

The evidence whether bLF is effective in preventing diarrhea in infants and toddlers is conflicting. It appeared to be clinically effective in a single study with 4- to 6-months-old infants who were followed over a 3-months period, but had shown no effect in 0- to 12- and 12- to 18-months-old infants and toddlers who were followed for 12 and 6 months, respectively.<sup>7-9</sup> Therefore, the effectiveness of bLF demonstrated by Chen et al.<sup>8</sup> could have been influenced in some way by the short duration of the study and by the especially narrow age group.

All three studies evaluated a large number of variables, and in two of them, diarrhea was not the primary outcome of the research.<sup>7-9</sup> Conducting a trial that focused on incidence rate of diarrhea in a narrow age group of infants or toddlers would be beneficial. Also, studying more than one dose of bLF in the same trial and increasing the doses as the participants grew would be useful. Doing so would leave less room for questioning whether a specific dose of bLF was most effective in diarrheal prevention for the evaluated age group. Additionally, wider range of the doses would provide the evidence is of a better quality.

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